CHARM SCIENCES, INC.
ROSA FAST AFLATOXIN QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA FAST Aflatoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. Aflatoxin is extracted from the sample using 70% methanol in water. Aflatoxin interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M reader and interpreted as parts per billion (ppb) aflatoxin.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	*Charm Sciences, Inc.* 978-687-9200
Test Kit Name:	ROSA FAST Aflatoxin Quantitative Test
Product Number:	LF-AFQ-FAST
Effective Date of Instructions:	05/01/2015
Instructions Revision Number	2
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, barley, brewer's rice, brown rice, corn flour, corn germ meal, corn gluten meal, corn meal, corn screenings, corn/soy blend, distillers dried grain, distillers dried grain with solubles, flaking corn grits, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, soybean hulls, soybean meal, soybeans, wheat, and wheat flour.
Extraction method:	Shake 50 gram sample with 100 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) for 1 minute. For **corn germ meal, distillers dried grain, distillers dried grain with solubles, soybean hulls, and soybeans**, add 150 mL 70% methanol/30% distilled or deionized water (v/v) and shake for 1 minute.
Test Format:	Lateral flow strip
Detection Method:	ROSA-M Reader, Model LF-ROSAREADER-M-NB Charm EZ-M reader, Model LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips:

Remove from the container only the number of test strips to be used in 1 day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours and unused test strips should be discarded.

b. AFQ Dilution Buffer:

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature (18 to 30 °C).

c. Preparation of Extraction Solvent [70% Methanol/30% Water (v/v)]:

The extraction solvent used in the method is a methanol/water mixture consisting of 70% methanol (reagent grade or better) and 30% distilled or deionized water (v/v).

(1) Using a 1000 mL graduated cylinder, measure 700 mL methanol and place it into a clean carboy with spigot.

(2) Using a 500 mL graduated cylinder, measure 300 mL distilled or deionized water and add to the methanol and shake until it is completely mixed.

(3) Label the container stating the mixture 70% methanol/30% water (v/v), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed. Mix again before use.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts distilled or deionized water.

d. Negative Control:

Prepare negative control by adding 100 microliters (µL) extraction solvent to 1.0 mL AFQ Dilution Buffer in a micro-centrifuge tube. Cap, mix and label.

e. Positive Control:

(1) Reconstitute the dry positive control (provided with test kit) by adding 300 µL extraction solvent followed by 3.0 mL AFQ Dilution Buffer. Mix well; allow to stand for 10 minutes at room temperature before use, and mix again just before use.

(2) Store reconstituted positive control refrigerated (0 to 7 °C) for up to 1 week or aliquot (at least 1.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution at -15 °C or below for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

f. Reader and Test Strip Performance Testing:

(1) Equipment Setup

(a) **ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting **AFLA SL** channel in 3-line mode (**AFLA SL** flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL).

(b) **Charm EZ-M Reader:** Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow Charm EZ-M reader prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select **AFQ-FAST (3 MIN)** from the TESTS list if prompted.

(2) Test calibration strips daily to verify ROSA-M Reader and Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges.

(3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:

(a) Negative Control: less than or equal to 2 ppb

(b) Positive Control: 12 to 28 ppb

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

g. ROSA Incubator:

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

EXTRACTION PROCEDURES

a. Procedure for corn, barley, brewer's rice, brown rice, corn flour, corn gluten meal, corn meal, corn screenings, corn/soy blend, flaking corn grits, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, soybean meal, wheat, and wheat flour:

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 100 mL extraction solvent.

(3) Shake vigorously for 1 minute (use within 30 minutes).

(4) Transfer 1 to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(5) Repeat for additional samples.

b. Procedure for corn germ meal, distillers dried grain, distillers dried grain with solubles, soybean hulls, and soybeans:

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 150 mL extraction solution.

(3) Shake vigorously for 1 minute (use within 30 minutes).

(4) Transfer 1 to 1.5 mL extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(5) Repeat for additional samples.

SAMPLE PREPARATION FOR QUANTIFICATION

This test kit uses different testing sensitivity ranges (Diluted Extract and Second Diluted Extract) for reporting aflatoxin measurements for grain and commodities.

a. <u>Sample Preparation of Diluted Extract or filtered Diluted Extract for 5 to 30 ppb quantitation:</u>

(1) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.

(2) Pipet 100 µL clarified extract to micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix (5 times inverting up and down), and label. This tube contains the Diluted Extract.

NOTE: For **barley, corn flour, corn gluten meal, corn meal, corn/soy blend, flaking corn grits, oats, popcorn, rye, wheat, wheat flour**, filter Diluted Extract using a Minisart RC15 syringe filter.

(a) Draw Diluted Extract into 1 mL syringe and pass through Minisart RC15 syringe filter.

(b) Collect the filtered Diluted Extract in a clean micro-centrifuge tube and label.

(3) Repeat for additional samples.

(4) Use Diluted Extract or filtered Diluted Extract (use within 6 hours after preparation) as your test sample in Sample Analysis found in Test Procedures section (page 5).

b. <u>Sample Preparation of Second Diluted Extract for 20 to 100 ppb quantitation:</u>

(1) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.

(2) Pipet 300 µL Diluted Extract or filtered Diluted Extract to micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix (5 times inverting up and down), and label. This tube contains the Second Diluted Extract.

NOTE: Laboratories may initially test the Second Diluted Extract if levels typically reported in their market area are within the 20 to 100 ppb testing range.

TEST PROCEDURES

a. <u>Sample Analysis:</u>

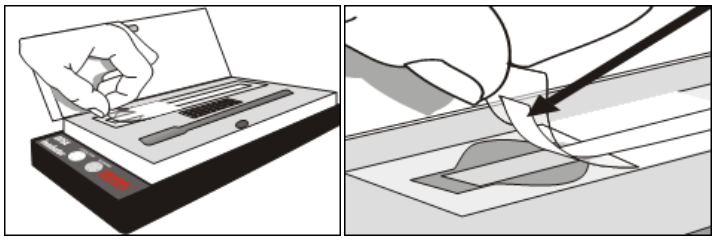
(1) Check that the ROSA Incubator temperature is 45 ± 1 °C.

(2) Label test strip(s) to identify sample.

(3) Place test strip in the ROSA Incubator with the flat side facing upward.

(4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to "Peel to Here" line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.



(5) Hold the pipet vertically and slowly pipet 300 µL test sample (Diluted Extract, Second Diluted Extract, Third Diluted Extract, Fourth Diluted Extract, or control) into the sample compartment at the ROSA Incubator line.

(6) Reseal the tape over the sample pad compartment.

NOTE: When performing multiple tests using a ROSA Incubator:

(a) Peel, pipet, and reseal before starting next strip.

(b) Complete all test strips within 1 minute.

(7) Close lid on the ROSA Incubator.

(8) Incubate test strip(s):

(a) Corn only and controls: **3 minutes**

(b) All other commodities: **5 minutes**

(9) Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

(a) Wipe foreign matter (dust, etc.) from the test strip(s).

(b) Inspect and read test strip within 2 minutes of incubation completion.

When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.

(c) Lower ROSA Incubator lid; do not re-latch.

b. <u>Visual Inspection:</u>

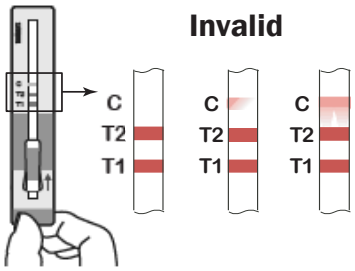
(1) The test strip is **INVALID** if any of the following are observed:

(a) C (Control) line is missing.

(b) T1, T2 (Test) or C line is smeared or uneven.

(c) T1, T2, or C line is obscured by diluted extract or control.

(d) Beads do not flow past T1, T2 or C lines.



(2) Do not put INVALID test strips in the ROSA-M Reader or Charm EZ-M reader.

(3) If test strip is INVALID, re-test the Diluted Extract, Second Diluted Extract, Third Diluted Extract, Fourth Diluted Extract, or control.

c. <u>Interpretation:</u>

(1) ROSA-M Reader

(a) Insert a clean and valid test strip into the ROSA-M Reader. Slide the strip into the slot with the sample compartment in the up position until it stops.

(b) Read results on **AFLA SL** channel in 3-line mode (**AFLA SL** flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.

1. For **corn**:

- **MATRIX 00:** Assay of Diluted Extract for 5 to 30 ppb quantitation.
- **MATRIX 01:** Assay of Second Diluted Extract for 20 to 100 ppb quantitation.
- **MATRIX 06:** Assay of Third Diluted Extract for 100 to 400 ppb (Corrected Aflatoxin) quantitation and assay of Fourth Diluted Extract for greater than 400 ppb (Uncorrected Aflatoxin) quantitation.

Refer to Supplementation Analysis section starting on Page 9 for preparation and analysis of Third Diluted Extract and Fourth Diluted Extract.

2. For **barley, brewer's rice, brown rice, corn flour, corn gluten meal, corn meal, corn screenings, corn/soy blend, flaking corn grits, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, soybean meal, wheat, wheat flour**:
   - **MATRIX 02:** Assay of Diluted Extract or filtered Diluted Extract for 5 to 30 ppb quantitation.
   - **MATRIX 03:** Assay of Second Diluted Extract for 20 to 100 ppb quantitation.
3. For **corn germ meal, distillers dried grain, distillers dried grain with solubles, soybean hulls, soybeans**:
   - **MATRIX 04:** Assay of Diluted Extract for 5 to 30 ppb quantitation.
   - **MATRIX 05:** Assay of Second Diluted Extract for 20 to 100 ppb quantitation.

For controls, see Reader and Test Strip Performance Testing in Preparation of Testing Materials and Equipment section (page 3).

(c) **READING:** The number displayed is the concentration of aflatoxin (ppb) in the sample.

A "+" sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range. For example, a Diluted Extract or filtered Diluted Extract READING of "+30 ppb" indicates a value greater than 30 ppb. For quantitation of 20 to 100 ppb aflatoxin, prepare the Second Diluted Extract and use with another test strip.

A Second Diluted Extract READING less than 20 ppb indicates a value below the detection range. Re-test Diluted Extract or filtered Diluted Extract on another test strip for quantitation from 5 to 30 ppb aflatoxin.

A Second Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. An applicant can request a supplemental analysis option (**corn only**) to report test results above the Second Diluted Extract sensitivity range of 100 ppb. See Supplement Analysis procedures for more information.

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the Diluted Extract or Second Diluted Extract test sample sensitivity ranges/concentrations.

(2) Charm EZ-M Reader

(a) Insert a clean and valid test strip into the Charm EZ-M. Slide the strip into the slot with the sample compartment in the down position until it stops.

(b) Read results on **AFQ-FAST (3 MIN)** for **corn only** and **AFQ-FAST (5 MIN)** for **all other commodities** from the TESTS list with COMMODITY and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.

- **DE:** Assay of Diluted Extract or filtered Diluted Extract for 5 to 30 ppb quantitation.
- **2ND DE:** Assay of Second Diluted Extract for 20 to 100 ppb quantitation.
- **3RD DE:** Assay of Third Diluted Extract for 100 to 400 ppb (Corrected Aflatoxin) quantitation. ***Refer to Supplemental Analysis section starting on Page 9 for preparation and analysis of Third Diluted Extract.***
- **4TH DE** Assay of Fourth Diluted Extract for 400 to 1600 ppb (Corrected Aflatoxin) quantitation. ***Refer to Supplemental Analysis section on starting on Page 9 for preparation and analysis of Fourth Diluted Extract.***

For controls, see Reader and Test Strip Performance Testing in Preparation of Testing Materials and Equipment section (page 3).

(c) **READING:** The number displayed is the concentration of aflatoxin (ppb) in the sample.

A "+" sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range. For example, a Diluted Extract or filtered Diluted Extract READING of "+30 ppb" indicates a value greater than 30 ppb. For quantitation of 20 to 100 ppb aflatoxin, prepare the Second Diluted Extract and use with another test strip.

A Second Diluted Extract READING less than 20 ppb indicates a value below the detection range. Re-test Diluted Extract or filtered Diluted Extract on another test strip for quantitation from 5 to 30 ppb aflatoxin.

A Second Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. An applicant can request a supplemental analysis option (**corn only**) to report test

results above the Second Diluted Extract sensitivity range of 100 ppb. See Supplement Analysis procedures for more information.

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the Diluted Extract, or Second Diluted Extract test sample sensitivity ranges/concentrations.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (**corn only**) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative aflatoxin test kits is 5 to 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. In supplemental analysis, the Second Diluted Extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range, and a correction for dilution is applied to derive the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental analysis performed on Third Diluted Extract for 100 to 400 ppb aflatoxin. For the ROSA-M Reader, the READING for Fourth Diluted Extract is an Uncorrected Aflatoxin Concentration in the sample and the Corrected Aflatoxin Concentration is obtained by multiplying the Uncorrected Aflatoxin Concentration by the dilution factor used to prepare the Supplemental Diluted Extract; for the Charm EZ-M reader, the appropriate calibration setting is selected for automatic correction for the supplemental analysis performed on Fourth Diluted Extract for 400 to 1600 ppb aflatoxin.

Supplemental analysis is performed only at the request of the applicant.

a. <u>Preparation and Assay of Third Diluted Extract for 100 to 400 ppb aflatoxin.</u>

(1) Prepare Second Diluted Extract according to Sample Preparation for Quantification section (page 4).

(2) Prepare Third Diluted Extract from the Second Diluted Extract for 100 to 400 ppb aflatoxin.

(a) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 300 µL Second Diluted Extract to micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the Third Diluted Extract.

(3) Repeat for additional samples.

(4) Use Third Diluted Extract as test sample in Sample Analysis found in Test Procedures section (page 5).

(5) Inspect and interpret the test strip as directed in Test Procedures section (page 6).

Valid Third Diluted Extract READING must be within 53 to 400 ppb detection range of the sample dilution.

A final result less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Second Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

A Third Diluted Extract READING of "+400 ppb" indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test result as greater than 400 ppb on the work record and certify "Aflatoxin exceeds 400 ppb". An applicant can request another supplemental analysis option (Fourth Diluted Extract) to report test results above the Third Diluted Extract sensitivity range of 400 ppb.

b. <u>Preparation and Assay of Fourth Diluted Extract for 400 to 1600 ppb aflatoxin.</u>

(1) Prepare Third Diluted Extract according to Supplemental Analysis section (page 9).

(2) Prepare Fourth Diluted Extract from the Third Diluted Extract for 400 to 1600 ppb aflatoxin.

(a) Pipet 300 µL AFQ Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 100 µL Third Diluted Extract to micro-centrifuge tube containing 300 µL AFQ Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the Fourth Diluted Extract.

(3) Repeat for additional samples.

(4) Use Fourth Diluted Extract as test sample in Sample Analysis found in Test Procedures section (page 5).

(5) Inspect and interpret the test strip as directed in Test Procedures section (page 6).

(a) ROSA-M Reader (Uncorrected Aflatoxin Concentration).

NOTE: The number/result displayed on the ROSA-M Reader is the Uncorrected Aflatoxin Concentration in the sample. Multiply the READING by four (4) to convert the Uncorrected Aflatoxin Concentration to the final Corrected Aflatoxin Concentration.

Examples: If the Uncorrected Aflatoxin Concentration is 200 ppb the final Corrected Aflatoxin Concentration is 800 ppb (200 ppb x 4 = 800 ppb). If the Uncorrected Aflatoxin Concentration is "+400 ppb" the final Corrected Aflatoxin Concentration is greater than 1600 ppb (+400 ppb x 4 = +1600 ppb).

Valid Fourth Diluted Extract READING must be within 209 to 1600 ppb detection range of the sample dilution.

A final result less than 209 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Third Diluted Extract (supplemental analysis) and only perform the supplemental analysis of Fourth Diluted Extract again if the value is greater than 400 ppb.

A Fourth Diluted Extract READING of "+1600 ppb" indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test results as greater than 1600 ppb on the work record and certify "Aflatoxin exceeds 1600 ppb".

(b) Charm EZ-M Reader (Corrected Aflatoxin Concentration).

Valid Fourth Diluted Extract READING must be within 209 to 1600 ppb detection range of the sample dilution.

A final result less than 209 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Third Diluted Extract (supplemental analysis) and only perform the supplemental analysis of Fourth Diluted Extract again if the value is greater than 400 ppb.

A Fourth Diluted Extract READING of "+1600 ppb" indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test results as greater than 1600 ppb on the work record and certify "Aflatoxin exceeds 1600 ppb".

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions:

(1) Store test strips refrigerated in tightly closed supplied container.

(2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.

(3) Store reconstituted positive control refrigerated (0 to 7 °C) for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution (-15 °C or below) for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b. Precautions:

(1) Test Strips

(a) To open test strip canister, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.

(b) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 25 to 30 minutes from the time the container was removed from the refrigerator.

(c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink

(2) Use AFQ Dilution Buffer supplied with each test kit only.

(3) Do not use the test kits beyond the noted expiration date.

(4) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.

(5) ROSA Incubator must be clean and level. ROSA Incubator temperature must be 45 ± 1 °C. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.

EQUIPMENT AND SUPPLIES

a. <u>Test Strips</u>

(1) LF-AFQ-FAST-20K

(a) 1 container of 20 AFQ-FAST test strips

(b) 1 Aflatoxin B1 Positive Control

(c) 1 AFQ Dilution Buffer

(2) LF-AFQ-FAST-100K

(a) 1 container of 100 AFQ-FAST test strips

(b) 1 Aflatoxin B1 Positive Control

(c) 2 AFQ Dilution Buffers

(3) LF-AFQ-FAST-500K

(a) 5 containers of 100 AFQ-FAST test strips

(b) 5 Aflatoxin B1 Positive Controls

(c) 10 AFQ Dilution Buffers

b. <u>Materials required but not provided</u>

(1) 100 µL pipet and pipet tips

(2) 300 µL pipet and pipet tips

(3) 1000 µL fixed volume pipet or 100 to 1000 µL variable volume pipet and pipet tips

(4) 100, 250, 500, and 1000 mL graduated cylinders

(5) Balance

(6) Deionized or distilled water

(7) Methanol (reagent grade or better)

(8) Micro-centrifuge tubes

(9) Mini-centrifuge

(10) ROSA-M Reader or Charm EZ-M reader

(11) Printer for ROSA-M Reader or Charm EZ-M reader (optional)

(12) ROSA Incubator

(13) Sample extraction Whirl-pak bags or containers

(14) Sample grinder

(15) Storage bottle

(16) Transfer pipets (optional)

c. <u>Materials required but not provided for barley, corn gluten meal, corn meal, corn/soy blend, flaking corn grits, oats, popcorn, rye, wheat, or wheat flour</u>

(1) Minisart RC15 syringe filters (Sartorius Minisart RC 15, Part No. 17762)

(2) Syringes

REVISION HISTORY

Revision 2 (05/01/2015)

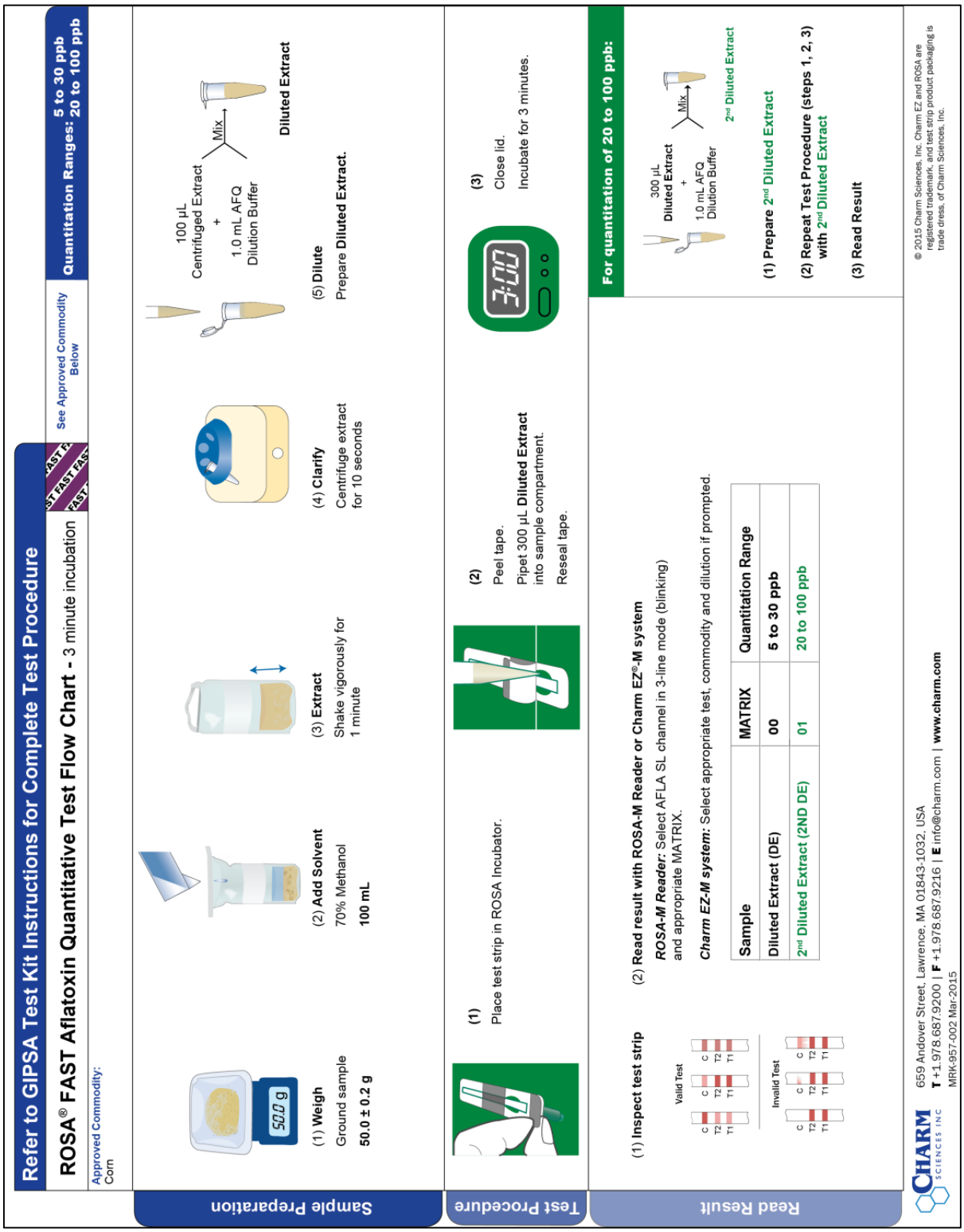
- Flow charts added.

Revision 1 (03/30/2015)

- Removed "settled" from the centrifugation step as the settling step has been removed.
- Add Fourth Diluted Extract to MATRIX 06 section for ROSA-M Reader on page 7.
- Supplemental Analysis section has been rearranged.
- The preparation of 70% Methanol has been updated to include using a 500 mL graduated cylinder.

Revision 0 (03/18/2015)

FLOW CHARTS


Refer to GIPSA Test Kit Instructions for Complete Test Procedure
ROSA® FAST Aflatoxin Quantitative Test Flow Chart - 3 minute incubation
See Approved Commodity Below
Quantitation Ranges: 5 to 30 ppb 20 to 100 ppb
Approved Commodity:
Corn
Sample Preparation
(1) Weigh
Ground sample
50.0 ± 0.2 g
50.0 g
(2) Add Solvent
70% Methanol
100 mL
(3) Extract
Shake vigorously for 1 minute
(4) Clarify
Centrifuge extract for 10 seconds
(5) Dilute
Prepare Diluted Extract.
100 µL Centrifuged Extract
+
1.0 mL AFQ Dilution Buffer
Mix
Diluted Extract
Test Procedure
(1)
Place test strip in ROSA Incubator.
(2)
Peel tape.
Pipet 300 µL Diluted Extract into sample compartment.
Reseal tape.
(3)
Close lid.
Incubate for 3 minutes.
3:00
Read Result
(1) Inspect test strip
Valid Test
Invalid Test
C
T2
T1
(2) Read result with ROSA-M Reader or Charm EZ®-M system
ROSA-M Reader: Select AFLA SL channel in 3-line mode (blinking) and appropriate MATRIX.
Charm EZ-M system: Select appropriate test, commodity and dilution if prompted.
Sample | MATRIX | Quantitation Range
Diluted Extract (DE) | 00 | 5 to 30 ppb
2nd Diluted Extract (2ND DE) | 01 | 20 to 100 ppb
For quantitation of 20 to 100 ppb:
300 µL Diluted Extract
+
1.0 mL AFQ Dilution Buffer
Mix
2nd Diluted Extract
(1) Prepare 2nd Diluted Extract
(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract
(3) Read Result
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MRK-957-002 Mar-2015
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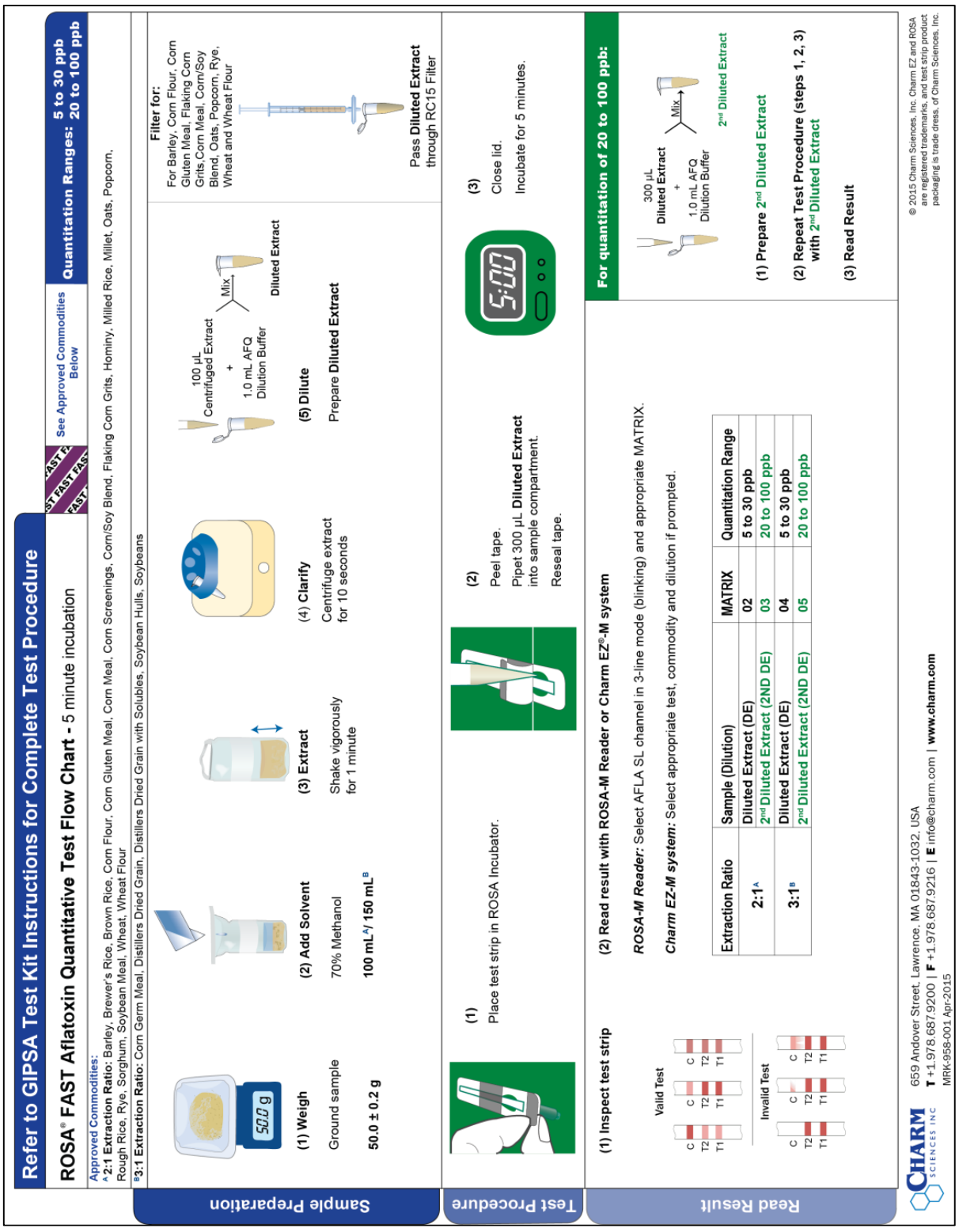
Refer to GIPSA Test Kit Instructions for Complete Test Procedure
ROSA® FAST Aflatoxin Quantitative Test Flow Chart - 5 minute incubation
See Approved Commodities Below
Quantitation Ranges: 5 to 30 ppb 20 to 100 ppb
Approved Commodities:
A 2:1 Extraction Ratio: Barley, Brewer's Rice, Brown Rice, Corn Flour, Corn Gluten Meal, Corn Meal, Corn Screenings, Corn/Soy Blend, Flaking Corn Grits, Hominy, Milled Rice, Millet, Oats, Popcorn, Rough Rice, Rye, Sorghum, Soybean Meal, Wheat, Wheat Flour
B 3:1 Extraction Ratio: Corn Germ Meal, Distillers Dried Grain, Distillers Dried Grain with Solubles, Soybean Hulls, Soybeans
Sample Preparation
500 g
(1) Weigh
Ground sample
50.0 ± 0.2 g
(2) Add Solvent
70% Methanol
100 mL A / 150 mL B
(3) Extract
Shake vigorously for 1 minute
(4) Clarify
Centrifuge extract for 10 seconds
(5) Dilute
Prepare Diluted Extract
100 µL Centrifuged Extract
+
1.0 mL AFQ Dilution Buffer
Mix
Diluted Extract
Filter for:
For Barley, Corn Flour, Corn Gluten Meal, Flaking Corn Grits,Corn Meal, Corn/Soy Blend, Oats, Popcorn, Rye, Wheat and Wheat Flour
Pass Diluted Extract through RC15 Filter
Test Procedure
(1)
Place test strip in ROSA Incubator.
(2)
Peel tape.
Piet 300 µL Diluted Extract into sample compartment.
Reseal tape.
(3)
Close lid.
Incubate for 5 minutes.
5:00
Read Result
(1) Inspect test strip
Valid Test
Invalid Test
C
T2
T1
(2) Read result with ROSA-M Reader or Charm EZ®-M system
ROSA-M Reader: Select AFLA SL channel in 3-line mode (blinking) and appropriate MATRIX.
Charm EZ-M system: Select appropriate test, commodity and dilution if prompted.
Extraction Ratio
Sample (Dilution)
MATRIX
Quantitation Range
2:1 A
Diluted Extract (DE)
02
5 to 30 ppb
2nd Diluted Extract (2ND DE)
03
20 to 100 ppb
3:1 B
Diluted Extract (DE)
04
5 to 30 ppb
2nd Diluted Extract (2ND DE)
05
20 to 100 ppb
For quantitation of 20 to 100 ppb:
300 µL Diluted Extract
+
1.0 mL AFQ Dilution Buffer
Mix
2nd Diluted Extract
(1) Prepare 2nd Diluted Extract
(2) Repeat Test Procedure (steps 1, 2, 3) with 2nd Diluted Extract
(3) Read Result
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MRK-958-001 Apr-2015
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